Effects of Cherries, Honeydew, and Bird Feces on Longevity and Fecundity of *Rhagoletis indifferens* (Diptera: Tephritidae)

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ABSTRACT In laboratory experiments, western cherry fruit flies, *Rhagoletis indifferens* Curran, were exposed to sweet cherries, aphid honeydew, and bird feces to determine fly longevity and fecundity. Longevity was not sustained in females and males exposed to intact unripe cherries or no food, whereas it was in females exposed to intact, fully-ripe cherries, and in females and males exposed to opened unripe or ripe cherries. Females exposed to intact fully-ripe cherries alone usually survived as long as females exposed to sucrose-yeast diets, but fecundity of flies exposed to cherries (79.5–110.8 eggs/female) was lower than that of flies exposed continuously to sucrose-yeast diets (277.5–326.2 eggs/female). Longevity of flies exposed to aphid honeydew was sustained and was similar to that of flies exposed to intact ripe cherries, sucrose-yeast, and sucrose diets. However, exposure to aphid honeydew or bird feces in the absence of ripe cherries did not result in high fecundity (4.6–32.2 eggs/female). Despite the inability of flies to extract nutrients from unripe cherries and the moderate fecundity of flies that were exposed to intact ripe cherries alone, *R. indifferens* was clearly capable of using its principal host fruit for both sustained longevity and egg production. Results suggest cherries damaged by birds throughout the season and intact fully-ripe cherries later in the season can contribute about the same nutrition as honeydew to female longevity, but that cherries contribute significantly more than either honeydew or bird feces to fecundity in nature.

KEY WORDS *Rhagoletis indifferens*, cherry, aphid honeydew, bird feces, fecundity

FEMALES OF THE WESTERN cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), have been observed feeding on the juice of sweet cherry, *Prunus avium* L., which they obtain by puncturing the fruit with their ovipositors (Frick et al. 1954, Mumtaz and AliNiazee 1983). Females and males also feed on juice that drips onto leaves after being opened by birds (W.L.Y., unpublished data). Despite documentation that feeding on cherry juice occurs in nature, it is not known if cherry juice provides adults with sufficient sugars for prolonged survival alone or with additional nutrients for sustained egg production.

Few studies have reported on the role of natural substances on *Rhagoletis* longevity (Middlekauf 1941) and fecundity (Neilson and Wood 1966), and only one, on the apple maggot, *Rhagoletis pomonella* (Walsh), has specifically studied the effects of exposure to fruit on longevity and fecundity (Hendrichs et al. 1993a). This study concluded that hawthorn fruit provided flies with carbohydrates in the form of leachates, materials removed from plants in aqueous solutions by the action of rain, dew, mist, and fog (Tukey and Morgan 1964, Tukey 1971). These substances apparently sustained moderate fecundity but not longevity in this species.

The only nutritional study on *R. indifferens* found that flies provided sucrose and cherries did not live as long as those exposed to sucrose alone and sucrose plus casein (Kamal 1954). Also, flies fed yeast and sucrose died slightly earlier than flies fed on sugar alone, presumably because protein (nitrogen) reduced longevity (Kamal 1954). Flies fed sucrose in the presence of cherries produced fewer larvae than flies fed sucrose alone, which produced as many as those fed sucrose plus casein. However, these results are inconsistent with the well-known positive effect of protein on egg development by tephritid fruit flies (Hagen 1953, Drew and Yuval 2000). In addition, the effects of cherries alone on longevity and fecundity in this study were not tested.

Rhagoletis indifferens is commonly found along with the black cherry aphid, *Myzus cerasi* (Fabricius) (Homoptera: Aphididae) within the same trees in the Pacific Northwest. When aphid infestations are high, copious amounts of honeydew are produced that drop on and contaminate leaves. *Myzus cerasi* is found on cherries from late March through mid June (Beers et al. 1993). The end of its life stages on cherry coincides with peak *R. indifferens* abundance. Unless washed off by rain, the aphid honeydew can remain on leaves as sticky, liquid, semiliquid, or dry deposits that can potentially serve as food for *R. indifferens* through June. Feces left behind by birds may also be a nutritional source for *R. indifferens*. Despite the presence of these

substances on cherry trees, it is not known if *R. indifferens* feeds on them, and if it does, whether fly fecundity is significantly enhanced. Fecundity of *R. pomonella* fed aphid honeydew and artificial diets were similar (Neilson and Wood 1966). Aphid honeydew and bird feces provided enough nutrition for moderate fecundity in *R. pomonella* (Hendrichs et al. 1993a).

Studies on tropical and subtropical fruit flies indicate opened fruit can contribute to sustained longevity and moderate fecundity (Hendrichs et al. 1993b, Jácome et al. 1999), as can honeydew (Hagen 1958). Bird feces alone do not contribute to longevity (Hendrichs et al. 1993b), but in the presence of carbohydrates, they may (Jácome et al. 1999) or may not (Thomas 1998) contribute to moderate fecundity.

The main objectives of this study were to determine whether cherries are a suitable food source for *R. indifferens* and how cherries, sucrose-yeast diets, aphid honeydew, and bird feces affect fly longevity and fecundity. Based on the fact that *R. indifferens* feeds on cherry juice in nature and the known roles of sugar and protein in other tephritids (Drew and Yuval 2000), three hypotheses with respect to cherries and sucrose-yeast diets were tested: (1) female flies exposed to intact cherries cannot survive as long as flies fed sucrose and yeast; (2) flies exposed to cherries alone lay fewer eggs than those exposed to sucrose and yeast; and (3) exposing flies first to sucrose and yeast alone then to cherries alone causes immediate reductions in both longevity and fecundity. I also hypothesized that feeding on aphid honeydew has similar effects on fly longevity, but not on fecundity, as feeding on feces and sucrose.

Materials and Methods

Source of Flies. Flies were collected as larvae from infested sweet and sour cherry fruit in Benton, Yakima, and Kittitas Counties, WA, in June and July 2000 and 2001. Pupae were stored in soil for 6–9 mo at 3°C, after which they were transferred to 27 ± 1°C. Adults were collected over 24-h periods after they emerged from the soil 3–4 wk later.

Experimental Conditions and General Methods. Unless noted, experiments were conducted using a single female-male pair per cage. Flies were placed inside 8.5 cm high × 8.5 cm wide paper carton cages with nylon organdy screen tops at 27–28°C under 16 h of 1,200–4,200 lux light and 50–70% RH. Deionized water was continuously provided in cotton wicks. Wicks were replaced periodically to prevent mold growth. In all experiments except experiment 1, only intact, dark purple, fully ripe sweet 'Bing' cherries (2–2.5 cm diameter, usually with green stems attached) that had been stored at 3°C for 1–4 mo were used. In all experiments, oviposition occurred either into a single cherry or on a single wax dome (2.5 cm diameter) made of orange wax (soft wax, Calwax Corp., Azusa, CA) (AliNiazee and Brown 1977). All eggs laid every 2 or 3 d were removed from fruit using jeweler's forceps after the skin of the fruit was peeled under a microscope. Eggs were laid ≈1 mm below the surface. Cherries and domes were replaced after each recording throughout the lifespan of the flies. Survival was checked each day. When a male died before the female, it was replaced with a new one, which served as a mate for the female.

Experiment 1. To determine the stages of cherries from which flies could obtain nutrients, flies were exposed to fruit of progressively increasing maturity. In test 1, 8–34 fly pairs reared in the laboratory were exposed to (1) water only, (2) green cherries, 1.0–1.2 cm diameter (collected 2 May), (3) yellow/orange cherries, 1.5–1.7 cm (22 May), (4) orange/red cherries, 2.0–2.3 cm (4–8 June), or (5) red cherries, 2.4–2.6 cm (11 June). Cherries were collected from uninfested trees in Benton and Yakima Counties, WA. In test 2, 4–10 pairs (replicates) of wild flies (ages unknown) collected from the field on 24 June were exposed to the following treatments: (1) water only, (2) intact orange/red cherries collected 8 June, and (3) intact fully-ripe, dark purple cherries collected 24 June. Some cherries in treatment 3 were from infested trees and had larval exit or respiration holes. In test 3, to simulate exposure of flies to fruit damaged by birds, 10–12 pairs of laboratory-reared flies were presented opened (1) yellow/orange, (2) orange/red, or (3) red cherries. Cherries were split with latex-gloved hands. Cherries were from uninfested trees. Green cherries were not used, because birds were not seen attacking them. Sugar content (Brix) of 10–12 cherries of each maturity stage was determined using a refractometer (Atago N1, Tokyo, Japan).

Experiment 2. To compare how access to cherries and sucrose-yeast diets affect longevity and fecundity, flies were exposed to cherries alone and to two sucrose-yeast diets in the presence of cherries. Sucrose-yeast diets were considered to be near optimal in nutrition, as fecundity of flies fed these was comparable to the maximum reported by Frick et al. (1954). A preliminary study showed that females exposed to dry 88% sucrose-12% yeast ($N = 2$) and wet 60% sucrose-12% yeast (wt:wt) ($N = 6$) diets survived 43.5 ± 10.5 and 43.7 ± 10.5 d (mean ± SE) and laid 195.5 ± 60.5 and 172.7 ± 62.7 eggs, respectively, at 25°C under laboratory conditions. These dry and wet sucrose-yeast diets thus provided strong comparisons with a cherry diet.

Treatments in experiment 2 were: (1) intact cherries alone throughout life; (2) surface-sterilized intact cherries alone throughout; (3) dry 88% sucrose-12% yeast diet throughout; (4) dry diet removed after 14 d, followed by cherries alone afterward; (5) wet 60% sucrose – 12% yeast diet throughout; (6) wet diet removed after 14 d, followed by cherries alone afterward; and (7) dry 100% sucrose alone throughout. All diets and sucrose were placed in small dishes. Cherries and diets were handled with latex gloves.

In treatment 2, cherries were soaked in a solution of 5.25% sodium hypochlorite (bleach) for 1.5 min, rinsed, and washed in deionized water to sterilize the surfaces. Sterilized cherries were replaced every 2 d. For treatments 3–6, diets were made of sucrose and

dry yeast extract (EZMix, Sigma Chemical Co., St. Louis, MO). The mixture was heated until it completely dissolved in solution and then dried or presented wet on cotton. Fresh, wet diets were introduced every 2 or 3 d. In treatments 3–6, cherries were placed into cages starting on day 8, around the time when oviposition first occurs (Frick et al. 1954). In treatment 7, a wax dome was provided for oviposition beginning on day 8. Domes were needed to separate effects of sucrose alone and cherry juice, but were not used in other treatments because oviposition into fruit, the natural behavior, was desired. There were 11–29 fly pairs (replicates) per treatment.

To determine whether feces from cherry-fed flies contributed to fecundity, nine fly pairs in a separate test were placed inside cages contaminated with fecal matter from previously cherry-fed flies. Dry sucrose alone was supplied as food. A wax dome was provided for oviposition.

Experiment 3. To provide more support for results from experiment 2 and to further determine the effects of cherry-feeding in relation to prior yeast-feeding on longevity and fecundity, a second experiment was conducted using three females and males each per cage. A dry 80% sucrose-20% yeast diet was used, as an earlier test had shown that females exposed to this diet survived 70.0 ± 5.4 d and laid 223.0 ± 43.2 eggs (N = 21) at 25°C. Treatments were (1) cherries alone throughout; (2) dry sucrose-yeast diet saturated in paper strips throughout; (3) dry diet removed after 14 d, followed by cherries alone afterward; (4) dry diet removed after 8 d, followed by cherries alone afterward; and (5) dry diet removed after 8 d, followed by no cherries afterward. Cherries were provided on day 8 in treatments 2–4. There were 5–10 groups of flies (replicates) for each treatment.

To assess viability of eggs produced by cherry- and diet (dry 80% sucrose-20% yeast)-fed flies, 200 eggs laid into fruit by 13–20-d-old females that had been caged with males were removed and transferred using a camel's-hair brush onto wet filter paper. Filter papers were placed inside air-tight Petri dishes at 27°C. Eggs were checked for hatch 4–8 d after they were laid.

Experiment 4. To determine the effects of *M. cerasi* honeydew and bird feces in the presence of cherries on longevity and fecundity, four treatments were included in two tests: (1) dry sweet cherry leaves only + cherry; (2) honeydew on leaves + cherry; (3) bird feces on leaves + cherry; and (4) honeydew and bird feces on leaves + cherry. In test 1, leaves were 5–10 cm^2 in area. In test 2, leaves were 25–50 cm^2. The larger areas increased the amount of honeydew and bird feces present. In both tests, the entire upper surfaces of leaves were covered with honeydew. In treatment 3 and 4, pieces of leaves either had intact individual feces that weighed 10–20 mg or feces that were thinly spread on the surface, covering ≈0.5–1.5 cm^2 and 1–3 cm^2 in tests 1 and 2, respectively. For each test, there were 10 females and 4–6 males (replicates) used for analyses per treatment. In some replicates, females were provided with males of unknown ages from the laboratory colony because same-aged males were not available. For the same reason, in other replicates males were provided with females of unknown ages. Thus there were unequal numbers of females and males used for analyses. This was also the case in experiments 5 and 6.

In experiment 4, honeydew, feces, and clean sweet cherry leaves had been collected during 8–11 July 2001 from trees that were naturally infested with *R. indifferens* in Yakima County, WA. Honeydew was collected from heavily contaminated leaves at lower levels from four trees in one yard. Bird feces were collected from three trees in another yard. Feces were probably from starlings, *Sturnus vulgaris* L., and American robins, *Turdus migratorius*, which were the most common birds feeding on the trees. All substances were stored at 27°C and 30–50% RH for 5–6 mo before experiments and were dry when used in experiments.

Experiment 5. Because the presence of cherries may have masked any benefits from honeydew, the effects of honeydew and sucrose in the absence of cherries on longevity and fecundity were also determined. Four comparisons were made: (1) leaves only, (2) 100% dry sucrose only, (3) leaves and dry 100% sucrose, and (4) leaves covered with honeydew. Sucrose was presented soaked and dried in cotton and separate from leaves. Bird feces were not available for this experiment. Aphid exoskeletons and dead aphids in treatment 4 adhering and embedded in honeydew were not removed. Leaves were 40–60 cm^2 and covered entirely (or nearly so) with honeydew. In all treatments, a wax dome was placed into a cage beginning at day 8. There were 11–17 females and 10–19 males (replicates) used per treatment.

Experiment 6. To further determine the effects of honeydew, sucrose, sucrose-yeast, and bird feces in the absence of cherries on longevity and fecundity, the following treatments were compared: (1) 100% dry sucrose only, (2) dry 80% sucrose-20% yeast, (3) fresh aphid honeydew, and (4) fresh bird feces. Fresh aphid honeydew was collected from five cherry trees in May and June 2002 and exposed immediately to flies, because of the possibility that older honeydew on leaves in experiments 4 and 5 differed in quality from fresh honeydew. Approximately 120–150 mg of honeydew were transferred from leaves onto clear plastic using flat-tipped forceps. Aphids and their exoskeletons were carefully removed. Fresh starling feces were also collected from around a nest in the field. Flies were exposed to 270–580 mg (dry weight) of feces. Feces were moistened twice a week. There were 12–20 females and 5–8 males (replicates) used per treatment.

Statistical Analyses. Total longevity and fecundity data were subjected to one-way analysis of variance (ANOVA), followed by the Scheffé procedure for multiple comparisons (Winer et al. 1991) ($P < 0.05$). In addition, in experiment 2, eggs were laid within three 12-d time periods – 8–20, 20–32, and 32–44 d after emergence. These numbers were analyzed separately to determine if there were effects of removing the yeast protein source and subsequent dependence

Table 1. Experiment 1: Effects of sweet 'Bing' cherry developmental stage (color, collection date) on longevity and fecundity of *Rhagoletis indifferens*. Single females were paired with single males

Treatment	Females			Males	
	N	Longevity (d) mean ± SE	Total no. eggs mean ± SE	N	Longevity (d) mean ± SE
Test 1 - Intact Cherries-Laboratory Flies					
1 Control	8	3.4 ± 0.3a	–	7	3.7 ± 0.2ab
2 Green, 2 May	13	3.8 ± 0.3a	–	12	4.0 ± 0.2b
3 Yellow/orange, 22 May	18	3.0 ± 0.2a	–	13	2.8 ± 0.1b
4 Orange red, 4–8 June	17	3.5 ± 0.2a	–	17	3.6 ± 0.1ab
5 Red, 11 June	34	4.2 ± 0.7a	–	35	4.3 ± 0.3a
Test 2 - Intact Cherries-Wild Flies[a]					
1 Control	4	2.0 ± 0.0a	–	5	2.4 ± 0.2b
2 Orange red, 8 June	6	6.2 ± 2.1a	–	4	2.5 ± 0.5ab
3 Dark purple[b], 24 June	10	20.8 ± 6.4a	–	9	4.1 ± 0.5a
Test 3 - Opened Cherries[c]-Laboratory Flies					
1 Yellow/orange, 22 May	10	6.9 ± 0.7b	0.0 ± 0.0b	9	7.4 ± 0.9b
2 Orange red, 4–8 June	12	17.4 ± 3.8ab	11.6 ± 5.5ab	13	27.2 ± 6.8ab
3 Red, 11 June	12	23.1 ± 2.7a	19.7 ± 9.7a	11	39.6 ± 8.1a

[a] Flies were collected from the field on 24 June; age at capture unknown.
[b] Fruit collected in the field 24 June; a few with larval respiration or small exit holes; fecundity not determined.
[c] Wax domes and opened cherries used as oviposition substrates.
Means followed by the same letter within tests and columns are not significantly different (Scheffé procedure, $P > 0.05$).

on cherries alone on fecundity. Egg counts were subjected to the square-root transformation. Analyses were performed using the Statistical Analysis System (SAS Institute 2001).

Results

Experiment 1. Females and males exposed to intact, unripe cherries (green to red) survived 2.4–6.2 d. Females exposed to intact, fully-ripe cherries survived 20.8 d, whereas males only survived 4.1 d. Females and males exposed to opened unripe and ripe cherries survived 6.9–39.6 d, with greater longevity for more mature cherries (Table 1). Fecundity of flies exposed to opened ripe (red) cherries was greater than that of flies exposed to unripe (yellow/orange) cherries. The sugar content of different stage cherries varied: it was not detectable in green cherries; for yellow/orange cherries, it was 5.4 ± 0.4%; orange red, 15.3 ± 0.6%; red, 19.6 ± 1.0%; and dark purple, 20.9 ± 0.7%.

Experiment 2. Females that were exposed to surface-sterilized cherries alone had lower longevity and fecundity than those exposed to the dry 88% sucrose-12% yeast and wet 60% sucrose-12% yeast diets throughout their lifetime. Females exposed to fully-ripe cherries also had lower fecundity than those exposed to sucrose-yeast (Table 2) (longevity: $F = 6.61$; df = 6, 99; $P < 0.0001$; fecundity: $F = 13.30$; df = 6, 99; $P < 0.0001$). When diets were removed after 14 d and cherries were the sole source of nutrition thereafter, longevity and fecundity were approximately one half that of the best diet (Table 2). Flies continuously exposed to cherries (not surface sterilized) alone and to sucrose and yeast laid eggs >60 d post emergence (Fig. 1). Females fed sucrose alone survived as long as cherry-fed flies, but laid fewer eggs (Table 2).

When data were analyzed by time periods, results differed from when data were summed over the lifetime. There were no differences in fecundity of any treatment at 8–20 and 20–32 d, but flies fed cherries alone laid fewer eggs from 32 to 44 d than flies exposed to the wet 60% sucrose-12% yeast diet throughout. Even after the sucrose-yeast diets were removed, eggs were laid at comparable levels to those seen when sucrose-yeast diets were never removed (Fig. 1).

Table 2. Experiment 2: Mean longevity and fecundity ± SE of female *Rhagoletis indifferens* exposed to fully ripe, intact sweet cherry, and dry and wet sucrose-yeast diets. Cherries were present in all treatments except 7. Single females were paired with single males

Treatment[a,b]	N	Longevity (d) mean ± SE	Total no. eggs mean ± SE	% Eggs of best diet
1 Ripe Cherry alone	29	33.1 ± 3.7ab	87.3 ± 16.4b	29.8
2 Surface-sterilized cherry	15	19.6 ± 4.3b	79.5 ± 22.8bc	27.2
3 Dry diet throughout	12	52.8 ± 7.3a	277.5 ± 57.6a	94.8
4 Dry diet for 14 d	11	35.4 ± 3.9ab	154.6 ± 36.3ab	52.8
5 Wet diet throughout	11	48.1 ± 5.8a	292.6 ± 49.5a	100.0
6 Wet diet for 14 d	11	31.8 ± 4.8ab	132.4 ± 42.9ab	45.2
7 Dry sucrose alone[c]	18	20.5 ± 2.6b	2.7 ± 1.9c	0.9

Means followed by the same letter within columns are not significantly different (Scheffé procedure, $P > 0.05$).
[a] Dry diet: 88% sucrose-12% yeast; wet diet: 60% sucrose-12% yeast.
[b] Cherries present from start in treatments 1 and 2; cherries introduced on day 8 in treatments 3–6.
[c] To prevent use of cherries as food, wax domes used for oviposition beginning on day 8.

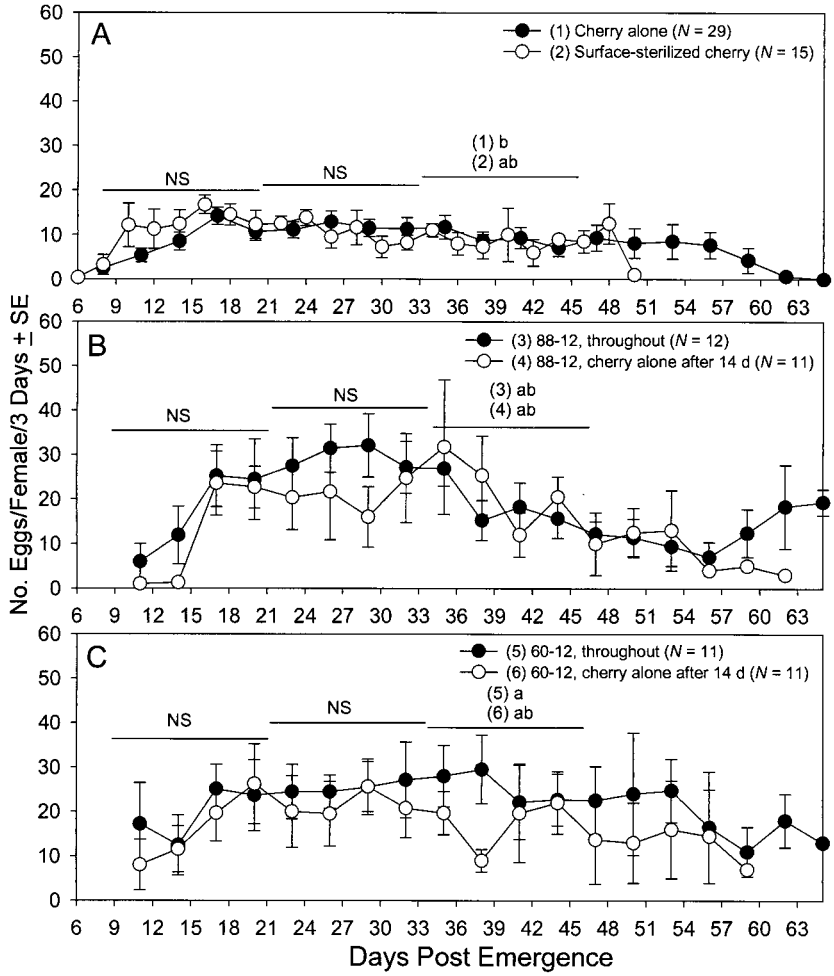

Fig. 1. Effects of (A) cherry, (B) 88% sucrose-12% yeast, and (C) 60% sucrose-12% yeast treatments on numbers of eggs laid by *Rhagoletis indifferens* over the lifetime. Horizontal lines indicate separately analyzed periods. Treatments within these periods across panels A-C followed by the same letter are not significantly different (Scheffé procedure, $P > 0.05$). NS = not significant.

Females caged with fly feces and exposed to sucrose alone survived 18.4 ± 2.6 d and laid only 0.6 ± 0.4 total eggs/fly. This suggested that feces did not positively affect egg production.

Experiment 3. Grouped females exposed to cherries and sucrose-yeast diets showed similar egg-laying patterns as the single flies. When flies were exposed to cherries alone, longevity and fecundity were less than that seen in flies exposed to the dry 80% sucrose-20% yeast diet throughout (longevity: $F = 14.54$; df = 4, 31; $P < 0.0001$; fecundity: $F = 6.39$; df = 3, 26; $P = 0.0022$) (Table 3). When diets were removed at 8 or 14 d and cherries were the sole source of nutrition thereafter, longevity and fecundity were approximately the same as for the cherry alone treatment. No difference was seen between 8- and 14-d exposures to the sucrose-yeast diet (Table 3). When the sucrose-yeast diet was removed at day 8 in the absence of cherries, flies only survived 10.1 ± 0.6 d ($P < 0.05$). Flies in all except the surface-sterilized cherry treatment laid eggs at >60 d post emergence (Fig. 2). There were no differences in fecundity of flies at 8–20 and 32–44 d, but flies fed cherries alone after 8 d laid fewer eggs from 20 to 32 d than flies exposed to the dry 80% sucrose-20% yeast diet throughout (Fig. 2).

Eggs laid by the cherry-fed flies were fertile, with 49.3% hatch. Eggs laid by sucrose-yeast fed females had a 37.0% hatch.

Experiment 4. When cherries were present, there were no differences between longevity and fecundity of females exposed to dry honeydew and feces on small (Test 1) or large leaves (Test 2) ($P > 0.05$), so data were pooled (Table 4). Longevity of females exposed to honeydew and feces was greater than in the control and feces treatment, although not statistically. Honeydew was still present on large leaves at the end of the experiments, indicating honeydew quantity did not limit nutrient acquisition. There were

Table 3. Experiment 3: Mean longevity and fecundity ± SE of female *Rhagoletis indifferens* exposed to fully ripe, intact sweet cherry, and dry and wet sucrose-yeast diets. Cherries were present in all treatments except 5. Three females were paired with three males

Treatment[a,b]	N	Longevity (d) mean ± SE	Total no. eggs mean ± SE	% Eggs of best diet
1 Ripe cherry alone	5	36.9 ± 4.9b	110.8 ± 14.9b	34.0
2 Dry diet throughout	8	59.9 ± 7.0a	326.2 ± 54.2a	100.0
3 Dry diet for 8 d	7	35.3 ± 3.1b	177.6 ± 21.5ab	54.4
4 Dry diet for 14 d	10	33.8 ± 3.2b	132.6 ± 27.6b	40.6
5 No cherry, dry diet for 8 d	6	10.1 ± 0.6c	–[c]	–[c]

Means followed by the same letter within columns are not significantly different (Scheffé procedure, $P > 0.05$).
[a] Dry diet: 80% sucrose-20% yeast.
[b] Cherries present from the start in treatment 1; cherries introduced on day 8 in treatments 2–4.
[c] No oviposition substrate provided.

no differences among males exposed to any treatment (Table 4).

Experiment 5. When cherries were absent, females exposed to dry honeydew in the presence of dead aphids on leaves lived longer ($F = 33.88$, df = 3, 48; $P < 0.0001$) and laid significantly more eggs ($F = 7.84$, df = 3, 48; $P = 0.0002$) than flies exposed to dry leaves alone, sucrose alone, and dry leaves + sucrose (Table 5). Dry leaves did not provide nutrients for prolonged longevity or fecundity. Males exposed to honeydew on leaves had greater longevity than males exposed to dry leaves alone or dry leaves + sucrose (Table 5).

Experiment 6. Females exposed to sucrose, sucrose and yeast, fresh honeydew, and fresh bird feces lived equally long in the absence of cherries ($P > 0.05$), but only the sucrose and yeast treatment resulted in any

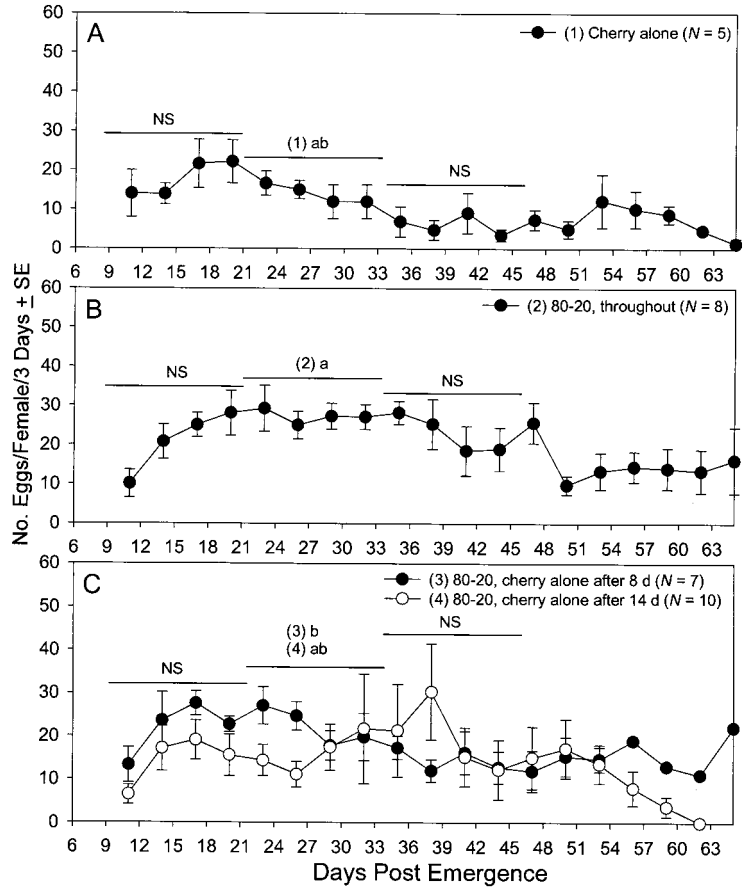

Fig. 2. Effects of (A) cherry, (B) 80% sucrose-20% yeast throughout, and (C) 80% sucrose-20% yeast-cherry alone treatments on numbers of eggs laid by *Rhagoletis indifferens* over the lifetime. Horizontal lines indicate separately analyzed periods. Treatments within these periods across panels A-C followed by the same letters are not significantly different (Scheffé procedure, $P > 0.05$). NS = not significant.

Table 4. Experiment 4: Effects of aphid honeydew and bird feces on longevity and fecundity of *Rhagoletis indifferens* in the presence of fully ripe, intact sweet cherries. Honeydew and feces covered 5 to 50 and 0.5–3 cm^2, respectively, on leaf surfaces. Single females were paired with single males

Treatment	Females			Males	
	N	Longevity (d) mean ± SE	Total no. eggs mean ± SE	N	Longevity (d) mean ± SE
1 Control leaves	20	35.5 ± 3.9a	139.4 ± 24.9a	12	21.8 ± 5.7a
2 Aphid honeydew	20	40.1 ± 4.0a	135.3 ± 25.0a	10	33.2 ± 5.8a
3 Bird feces	20	31.9 ± 4.0a	175.0 ± 29.2a	9	18.9 ± 4.6a
4 Aphid honeydew + bird feces	20	49.9 ± 5.7a	199.9 ± 33.0a	11	41.3 ± 7.8a

Means followed by the same letter within columns are not significantly different (Scheffé procedure, $P > 0.05$).

appreciable fecundity ($F = 12.93$; df = 3, 56; $P < 0.0001$) (Table 6). Unlike in experiment 5, fresh honeydew without aphids on plastic did not contribute to fecundity any more than sucrose alone. After flies died, there was still 73 ± 12 mg ($N = 7$) of honeydew left on the plastic. There were no differences in male longevity among treatments (Table 6).

Discussion

Rhagoletis indifferens was unable to extract adequate nutrients from intact green to red cherries, which are more difficult to penetrate than ripe fruit (Messina et al. 1991). Females apparently can insert their ovipositors and lay eggs into green immature fruit (Frick et al. 1954, Raine and Andison 1958), suggesting the inability to survive on intact unripe fruit is caused in part by the lack of or low volume of juice and sugar that exude from oviposition or feeding stings. In the Yakima Valley of Washington, flies emerge in late May, when fruit are still green, and probably cannot use intact cherries as food until they ripen in mid to late June. Intact ripe cherries that are fed upon during the harvest and postharvest periods [when some flies are still emerging (Frick et al. 1954)] could contribute to larval production before fruit become overly ripe, drop off the tree, or dry 2–3 wk later. Unlike intact cherries, opened cherries can play a valuable role in fly nutrition much earlier in the season. Birds attack cherries any time after the yellow/orange to red stage, in many cases leaving much of the exposed flesh attached to the pits. Cherries also split and crack after heavy rain, although feeding in these cherries has not been observed. Feeding on wounds made by birds is probably a major way the flies obtain nutrients from cherries in nature. This may be especially true for males, which cannot pierce fruit, explaining the inability of males to sustain themselves on almost all stages of intact fruit.

No other *Rhagoletis* species has been reported to achieve both sustained longevity and fecundity on intact or opened fruit, although several *Rhagoletis* species have been observed using their larval developmental host as an adult food source. Feeding on cherry fruit juice (on intact or damaged fruit) occurs in the black cherry fruit fly, *R. fausta* (Osten Sacken) (Frick et al. 1954), European cherry fruit fly, *R. cerasi* L. (Leski 1963), and the eastern cherry fruit fly, *R. cingulata* (Loew) (Smith 1984), and on *Solanum* spp. in *R. conversa* (Bréthes) (Frías et al. 1984). *Rhagoletis berberis* Curran punctures Oregon grape, *Mahonia aquifolium* Paxton, and feeds on its juices (Mayes and Roitberg 1986). In addition, *R. suavis* (Loew) feeds on exudates from oviposition punctures in walnuts (Bush 1966). *Rhagoletis pomonella* apparently does not obtain many nutrients from intact or opened apples (Hendrichs et al. 1993a). Other tephritids can sustain longevity from nutrients in opened fruit, but fecundity is less than on sugar-yeast diets (Table 7).

The hypothesis that female *R. indifferens* exposed to intact cherries alone can not survive as long as flies fed sucrose and yeast was supported. Nevertheless, *R. indifferens* was able to survive surprisingly long on intact cherries alone. Glucose and fructose are the major sugars in sweet cherries (Girard and Kopp 1998) and were likely the key nutritional constituents that sustained fly longevity. Females of *R. indifferens* have been seen puncturing fruit 6 d before oviposition (Frick et al. 1954), suggesting this was a feeding behavior.

The hypothesis that *R. indifferens* exposed to cherries alone lay fewer eggs than those exposed to sucrose

Table 5. Experiment 5: Effects of dry leaves, sucrose, and aphid honeydew on longevity and fecundity of *Rhagoletis indifferens* in the absence of cherries.[a] Honeydew covered 40–60 cm^2 on leaf surfaces. Single females were paired with single males

Treatment	Females			Males	
	N	Longevity (d) mean ± SE	Total no. eggs mean ± SE	N	Longevity (d) mean ± SE
1 Dry leaves	17	3.8 ± 0.2c	0.0 ± 0.0b	10	3.2 ± 0.2c
2 Dry sucrose	12	20.4 ± 3.5b	1.3 ± 1.0b	19	30.5 ± 3.9ab
3 Dry leaves + dry sucrose	12	13.7 ± 2.5b	1.1 ± 0.6b	11	21.8 ± 3.3b
4 Aphid honeydew on leaves	11	36.5 ± 3.1a	32.2 ± 12.6a	11	36.6 ± 4.8a

Means followed by the same letter within columns are not significantly different (Scheffé procedure, $P > 0.05$).
[a] Wax domes used as oviposition substrate.

Table 6. Experiment 6: Effects of sucrose foods, fresh aphid honeydew, and fresh bird feces on longevity and fecundity of *Rhagoletis indifferens* in the absence of cherries.[a] Single females were paired with single males

Treatment	Females			Males	
	N	Longevity (d) mean ± SE	Total no. eggs mean ± SE	N	Longevity (d) mean ± SE
1 Dry sucrose	12	30.2 ± 4.1a	3.9 ± 1.6b	5	21.4 ± 8.0a
2 80% sucrose-20% yeast	10	38.7 ± 7.4a	102.2 ± 32.5a	9	31.9 ± 10.3a
3 Fresh honeydew on plastic	18	25.7 ± 5.0a	10.3 ± 4.7b	5	32.2 ± 5.6a
4 Fresh bird feces + sucrose	20	27.9 ± 4.1a	4.6 ± 2.5b	8	32.9 ± 8.3a

Means followed by the same letter within columns are not significantly different (Scheffé procedure, $P > 0.05$).
[a] Wax domes used as oviposition substrate.

and yeast (continuously) was also supported, indicating a protein source other than cherries is needed to maximize this parameter. One gram of yeast extract contains 10.9 g total nitrogen (Sparks 1998) [or 68.1% protein, total nitrogen × 6.25, using the Kjeldahl procedure (Maynard et al. 1979)], whereas one g of sweet cherry flesh contains 0.012 g protein (1.2% by fresh weight) (USDA 1999a). As a percentage of food weight, the protein content of the yeast-sucrose foods in this study was 8.2–13.6%, or 6.8–11.3 times higher than in cherries. In *R. pomonella*, intact hawthorn fruit in the presence of sucrose also provided nutrients for only low egg production (Hendrichs et al. 1993a). Other nutrients in sweet cherries that may positively affect longevity and fecundity include vitamins C, B6, B12, calcium, phosphorus, pantothenic acid, and potassium (complete nutrient list, see USDA 1999b). In the oriental fruit fly, *Bactrocera dorsalis* (Hendel),

Table 7. Summary of major results of other studies on effects of fruit, honeydew, and bird feces on longevity and fecundity in relation to sugar (low quality) and sugar-yeast (high quality) diets[a] in tephritid fruit flies pertinent to current study

Food	Fly species	Food source, longevity results[a]	Reference
Intact fruit			
	R. pomonella	apple, > leaves	Neilson and Wood (1966)
	R. pomonella	hawthorn, < sucrose, sucrose-yeast	Hendrichs et al. (1993a)
	A. serpentina	sapodilla, < sucrose, sucrose-yeast	Jácome et al. (1999)
Opened fruit			
	R. pomonella	hawthorn (+ sucrose), = sucrose, sucrose-yeast	Hendrichs et al. (1993a)
	C. capitata	figs, = sucrose-yeast	Hendrichs et al. (1993b)
	C. capitata	grapes, = sucrose, sucrose-yeast	Hendrichs et al. (1993b)
	A. serpentina	sapodilla, >sucrose only, = sucrose-yeast	Jácome et al. (1999)
Honeydew			
	R. pomonella	aphid, < honey-yeast	Middlekauf (1941)
	R. pomonella	aphid, > leaves	Neilson and Wood (1966)
	R. pomonella	aphid (+ sucrose), = sucrose, sucrose-yeast	Hendrichs et al. (1993a)
	A. ludens	scale insect, = sucrose	Hagen (1958)
Bird feces			
	R. pomonella	unknown birds (+ sucrose), = sucrose	Hendrichs et al. (1993a)
	C. capitata	unknown birds (+ figs), = sucrose-yeast	Hendrichs et al. (1993b)
	A. serpentina	primaveras (+ sucrose), = sucrose, sucrose-yeast	Jácome et al. (1999)

Food	Fly species	Food source, fecundity results[a]	Reference
Intact fruit			
	R. pomonella	hawthorn, = sucrose	Hendrichs et al. (1993a)
	A. serpentina	sapodilla, < sucrose, sucrose-yeast	Jácome et al. (1999)
Opened fruit			
	R. pomonella	hawthorn (+ sucrose), = sucrose, sucrose-yeast	Hendrichs et al. (1993a)
	C. capitata	figs, < sucrose-yeast	Hendrichs et al. (1993b)
	C. capitata	grapes, = sucrose, < sucrose-yeast	Hendrichs et al. (1993b)
	A. serpentina	sapodilla, > sucrose, < sucrose-yeast	Jácome et al. (1999)
Honeydew			
	R. pomonella	aphids, > leaves	Neilson and Wood (1966)
	R. pomonella	aphids (+ sucrose), > sucrose, <sucrose-yeast	Hendrichs et al. (1993a)
	D. dorsalis	scale insect, = carbohydrates-yeast	Hagen (1958)
	A. ludens	scale insect, >fructose, < fructose-yeast	Hagen (1958)
Bird feces			
	R. pomonella	unknown birds (+ sucrose), = sucrose, <sucrose-yeast	Hendrichs et al. (1993a)
	C. capitata	unknown birds (+ figs), < sucrose-yeast	Hendrichs et al. (1993b)
	A. ludens	dove (+ sucrose), = sucrose	Thomas (1998)
	A. serpentina	primaveras (+ sucrose), > sucrose, <sucrose-yeast	Jácome et al. (1999)

Fly genera: *R., Rhagoletis; C., Ceratitis; A., Anastrepha; D., Dacus.*
[a] Some studies had no comparisons with either or with only one of the two. =, <, >: same as, lower than, or higher than on specified sugar and/or sugar-yeast diet.

adding B vitamins and minerals to a diet of sugar and protein increased longevity and were essential (especially choline) for öogenesis (Hagen 1953).

Juices that leached to the cherry fruit surface may have had higher sugar and protein concentrations (as a result of water evaporation) and therefore more nutrition than juice that oozed directly from punctures in fruit. Extensive grazing behavior on fruit surfaces was observed and was similar to grazing behavior on leaf surfaces, where carbohydrates are known to be present in many plants (Tukey and Morgan 1964, Tukey 1971). It is unknown how effective grazing on fruit compares with grazing on leaves in obtaining nutrients. In *R. indifferens,* juice rather than bacterial or other microbial growth on the cherries was almost certainly the source of nutrition, based on the results with surface-sterilized cherries. Leaf surface bacteria did not support significant egg production in *R. pomonella* (Hendrichs et al. 1993a), although bacteria did in tropical *Dacus* spp (Drew et al. 1983). Like *R. indifferens, R. pomonella* was apparently able to obtain some nutrients (leachates) from the intact fruit surface, although these nutrients were insufficient for prolonged survival (Hendrichs et al. 1993a).

The hypothesis that exposing flies first to sucrose and yeast alone then to cherries alone causes immediate reductions in longevity and fecundity was partly supported. Removal of sucrose-yeast diets resulted in decreased longevity (experiment 3), suggesting protein in yeast had no deleterious effect on the longevity of *R. indifferens,* similar to observations with some tephritids (Fluke and Allen 1931, Jácome et al. 1999), but unlike previous results with *R. indifferens* (Kamal 1954) and other species (Boyce 1934, Tsiropoulos 1983). In *R. indifferens,* removal of the sucrose-yeast diet resulted in consistently lower fecundity (although usually not significant), probably in part because of decreased longevity on a diet of cherries alone. However, effects of sucrose-yeast removal were not immediate (at 8–20 d), indicating conversion of extra protein to aid in egg development was delayed until after this time. Flies from which yeast diet had been removed were able to lay eggs at levels similar to flies with continuous yeast diet at 20–32 d. This suggests flies were positively affected by prior protein feeding. As long as cherries were present, the protein in the fruit and that obtained from the yeast could be used to develop eggs that were laid ≈12 d later. Perhaps some early egg development in *R. indifferens* resulted from nutrients carried over from the larval stage, as in *Ceratitis capitata* (Wiedemann) (Galun et al. 1981).

Green cherries are not a food source for *R. indifferens* early in the season, but aphid honeydew may be a sugar source for both females and males because it is readily available on leaves when flies first emerge in late May. Honeydew clearly provided adequate sugars that sustained longevity in both females and males (compared with unfed and sucrose-fed flies), as in other tephritids (Table 7). In *R. indifferens,* fecundity was moderate, but significantly increased (compared with sucrose controls), when flies were exposed to old honeydew with dead aphids and aphid exoskeletons on leaves in the absence of cherries. However, it was insignificant when flies were exposed to fresh honeydew on plastic. Microorganisms may have grown on the old honeydew or body fluids from dead aphids had mixed with the honeydew, resulting in higher protein ingestion by the flies and confounding effects of the honeydew itself. In *R. pomonella* and tropical tephritids, honeydew, when compared with sugar-yeast diets, also had no or only moderate effects on fecundity (Table 7).

The hypothesis that feeding on aphid honeydew has similar effects on longevity but not on fecundity of females as feeding on feces and sucrose was partly supported. Exposure to honeydew and bird feces + sucrose resulted in equal longevity. However, bird feces + sucrose did not increase fecundity. Thus bird feces, like aphid honeydew (at least in the absence of dead aphids and exoskeletons), were not a valuable source of protein or nitrogen for egg production. In other tephritids, bird feces, when compared with sugar or sugar-yeast diets, also had no or only moderate positive effects on fecundity (Table 7).

In summary, despite the inability of *R. indifferens* to extract nutrients from unripe fruit and the moderate fecundity of flies that were exposed to intact ripe cherries alone, *R. indifferens* was clearly capable of using its principal host fruit for both sustained longevity and egg production. Early in the season, cherries damaged by birds can provide an important source of nutrition for *R. indifferens.* Later in the season, these cherries and intact fully-ripe cherries, in addition to those with larval exit or respiration holes, may play the same role. Nutrients from cherries contribute the same as honeydew to female longevity, but cherries contribute more than either aphid honeydew or bird feces to fecundity. Other natural foods not yet identified probably play more important roles than honeydew and bird feces (which resulted in only 4.6–32.2 eggs per female) in the nutrition of *R. indifferens.* As proposed for *R. pomonella* (Hendrichs et al. 1993a), leaf leachates (Tukey 1971) are primary candidates. Their roles and those of microorganisms (especially yeast) growing on leaves in affecting the longevity and fecundity of *R. indifferens* need to be investigated.

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